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博 士 学 位 论 文

有机锡对褐菖鲉性腺发育和胚胎发育的影响

Effect of Organotin Compounds on Gonad Development and
Embryo Development in *Sebastiscus marmoratus*

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厦门大学博硕士论文摘要库

摘 要

有机锡化合物,特别是三丁基锡(tributyltin, TBT)和三苯基锡(triphenyltin, TPT),被广泛用于海洋船只防污涂料。有机锡致使软体动物性畸变已开展了较多的研究,然而,国际上有机锡对鱼类生殖和发育的研究才刚刚开始,其产生作用的机制尚不清楚。本实验以我国海洋经济鱼类褐菖鲉(*sebastiscus marmoratus*)为对象,研究环境水平 TBT (1、10 和 100 ng/L) 和 TPT (1 和 10 ng/L)) 对性腺发育的影响以及 TBT (0.01、0.1、1 和 10 ng/L) 对胚胎发育的影响。

TBT 暴露雄性成体褐菖鲉 48 d 后,尽管精巢游离睾酮(testosterone, T)水平增加,并且游离 17 β -雌二醇(17 β -estradiol, E2)水平降低,精子的发生却被抑制,表现为性腺指数(gonadosomatic Index, GSI)的降低、成熟精子数目的减少和较早期精母细胞数目的增加。TBT 暴露还抑制了被用作精巢支持细胞标志物的 γ -谷氨酰转肽酶(γ -glutamyl transpeptidase, γ -GTP)活性,同时在 100 ng/L TBT 暴露组的褐菖鲉精巢小叶间隙观察到严重的纤维化现象。另外,我们发现 TBT 诱导精巢内视黄类受体(retinoid X receptors, RXRs)和过氧化物酶体增殖物激活受体 γ (peroxisome proliferators activated receptor γ , PPAR γ) mRNA 的表达,增加精小叶间隙脂滴数量。本实验还发现,各 TBT 暴露组雌激素受体 α (Estrogen receptor α , ER α) mRNA 表达水平较对照组均有显著性降低,表现出剂量依赖性抑制降低。我们认为精巢内由 E2 水平降低导致的 ER α mRNA 表达水平的降低和精巢支持细胞上脂质的异常累积可能影响支持细胞的正常功能,从而影响精巢的发育。

TBT 暴露雌性成体褐菖鲉 48 d 后,卵巢游离 T 水平增加,而游离 E2 水平降低。同时, TBT 暴露抑制了卵巢的发育,表现为 GSI 的降低以及卵巢中卵黄期滤泡数量减少。另外, TBT 暴露还诱导了卵巢组织间隙大量异常脂质累积,但减少卵巢滤泡卵黄内的脂质小滴的数量。由于 TBT 阻止了卵黄对脂质吸收和累积,从而抑制了卵巢的发育。

TBT 暴露成体褐菖鲉 48 d 后,端脑皮层、视盖颗粒层和小脑区域的脑细胞凋亡数目增加。另外, TBT 增加脑组织活性氧(reactive oxygen species, ROS)和一氧化氮(nitric oxide, NO)的水平,抑制脑组织 Na⁺, K⁺-ATP 酶活性。这些结果表

明环境水平 TBT 能够导致脑损伤。鱼脑的功能与其生殖行为和性激素分泌具有密切的联系,因此脑损伤可能是 TBT 抑制性腺发育的一种重要机制。TBT 还能够抑制脑内 C II GnRH 基因转录。C II GnRH 基因转录水平的降低可能影响垂体-性腺轴,从而影响性腺的发育。

TBT 暴露成体褐菖鲉 48 d 后,引起了严重的甲状腺组织损伤,降低了血清三碘甲腺原氨酸 (3-5-30-triiodo-L-thyronine, T3) 和甲状腺素 (L-thyroxine, T4) 水平,抑制了精巢内甲状腺受体 α (thyroid hormone receptor α , TR α) mRNA 表达。由于甲状腺机能在性腺发育中的重要作用, TBT 对甲状腺机能的影响可能影响褐菖鲉性腺发育。

TPT 暴露成体褐菖鲉 48 d 后,精巢和卵巢的发育被抑制,精巢和卵巢内脂质水平增加,精巢和卵巢游离 T 水平上升,而游离 E2 水平降低。和 TBT 相比, TPT 具有相似的效应,但毒性要高于 TBT。

另外,本研究还以褐菖鲉胚胎为对象,研究 TBT 对胚胎发育的影响。结果表明, TBT 抑制褐菖鲉胚胎的出膜率,诱导明显的形态畸形,包括脊柱弯曲和心包囊水肿。另外,在脊柱弯曲胚胎的尾端出现“S”形弯曲畸形和细胞凋亡增加。TBT 暴露还导致严重的躯干部损伤,表现为骨骼肌细胞排列紊乱、凋亡细胞增加和乙酰胆碱酯酶活性的增加。TBT 抑制了 Na⁺,K⁺-ATP 酶、Ca²⁺-ATP 酶和碱性磷酸酶的活性,改变了 RXR、RAR (retinoic acid receptor, RAR)、TR、GR (glucocorticoid receptor, GR)、VDR (vitamin D receptor, VDR) 和 shh (sonic hedgehog, shh) mRNA 在胚胎中的表达。这为了解有机锡对鱼类胚胎发育毒性效应及其机制进行了有益的探索。

综上所述,本研究的结果表明,环境污染水平的有机锡抑制了褐菖鲉成鱼的精巢和卵巢的发育,这个效应与已有的关于有机锡对仔鱼的效应是不同的。实验结果为全面了解有机锡产生鱼类生殖毒性效应、途径和机制提供了新的重要的资料;为解释 TBT 的生殖毒性效应提供了新的机制上的理解。环境水平的 TPT 能够抑制精巢和卵巢的发育,而且毒性要高于 TBT;环境污染水平的 TBT 会影响褐菖鲉胚胎的成活和发育。这些结果为海洋环境政策的制定,为评估有机锡污染对海洋鱼类资源的影响提供了重要的依据。

关键词: 三丁基锡 褐菖鲉 性腺发育 胚胎发育 机制

ABSTRACT

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT) which have been used as antifouling biocides, can induce masculinization in female mollusks. However, few studies addressing the effects and mechanisms of organotin compounds on reproduction and development of fish have been reported. The present study was conducted to investigate the effects of TBT (1, 10, and 100 ng/L) and TPT (1 and 10 ng/L) on gonad development, and TBT (0.01, 0.1, 1, and 10 ng/L) on embryo development in *Sebastiscus marmoratus*.

After TBT exposure to male adult *S. marmoratus* for 48 d, the testes development was inhibited, which was shown as a decrease of gonadosomatic index (GSI) and a reduced number of mature sperm and an abundance of the late stages of spermatocysts in the testes. Although the free testosterone (T) levels in the testes were elevated and the free 17 β -estradiol (E2) levels were decreased, spermatogenesis was suppressed. Moreover, γ -glutamyl transpeptidase activity (γ -GTP) which is used as a Sertoli cell marker was decreased in a dose-dependent manner after TBT exposure, and serious interstitial fibrosis was observed in the interlobular septa of the testes in the 100 ng/L TBT group. Increases in the retinoid X receptors (RXRs) and peroxisome proliferators activated receptor γ (PPAR γ) mRNA expression and the progressive enlargement of lipid droplets in the testes were observed after TBT exposure. Estrogen receptor α (ER α) levels in the testes of the fish exposed to TBT decreased in a dose-dependent manner. The reduction of ER α mRNA resulted from the decrease of free E2 levels, and the progressive enlargement of lipid droplets may have contributed to the dysfunction of the Sertoli cells, which then disrupted spermatogenesis.

After TBT exposure to female adult *S. marmoratus* for 48 d, the free T levels in the testes were elevated and the free E2 levels were decreased. In addition, the ovaries development was inhibited, which was shown as a decrease of GSI and a reduced number of vitellogenic stage follicles in the ovaries. Moreover, TBT induced an

enlargement of interstitial lipid droplets in the ovaries, but decreased lipid droplets in the vitelline of follicles. Because TBT can prevent the vitelline from absorbing and accumulating lipid, the ovaries development was inhibited.

After TBT exposure to adult *S. marmoratus* for 48 d, apoptosis in brain cells of three regions including the pallial areas of the telencephalon, the granular layer of the optic tectum, and the cerebellum was induced. In addition, the increase of reactive oxygen species (ROS) and nitric oxide (NO) levels, and the decrease of Na^+/K^+ -ATPase activity were found in the brain. The results strongly indicated neurotoxicity of TBT to fishes. According to the important roles of brain in reproductive behavior and sex hormones secretion, the brain damage induced by TBT exposure might be one of the mechanisms affecting the gonad development. In the present study, TBT also inhibited the expression of C II GnRH in brain, which might affect the pituitary-gonad axis and then inhibited the gonad development.

After TBT exposure to adult *S. marmoratus* for 48 d, the thyroid status revealed severe damage of thyroid gland, decreased triiodothyronine (T3) and thyroxine (T4) in the serum and low expression of thyroid hormone receptor α (TR α) mRNA in the testes after TBT exposure. Because thyroid plays an important role in gonad development, the inhibition of thyroidal status induced by TBT might be one of the mechanisms affecting the gonad development.

After TPT exposure to adult *S. marmoratus* for 48 d, the testes and ovaries development was inhibited, free T levels in the testes and ovaries were elevated while the free E2 levels were decreased. Moreover, the total lipid in the testes and ovaries was increased. The effects of TPT are similar to TBT, but more poisonous than TBT.

We also examined the developmentally toxic of TBT on *S. marmoratus* embryo. Our study showed that TBT reduced the hatchability and caused apparent morphological abnormalities (including dorsal curvature and pericardial edema). In addition, a severely twisted tail and localized apoptosis was found in the embryos with dorsal curvature after TBT exposure. TBT exposure also caused trunk damage which was shown as the somatic muscle disorder, and the increase of apoptotic somatic cells and acetylcholinesterase activities. TBT depressed the Na^+,K^+ -ATPase,

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